ASED http://e-ASED.org
Animal Systematics, Evolution and Diversity

Anim. Syst. Evol. Divers. Vol. 35, No. 2: 84-90, April 2019
https://doi.org/10.5635/ASED.2019.35.2.011

Short communication

A New Record of the Brittle Star, *Amphistigma minuta* (Ophiuroidea: Amphilepidida: Amphiuridae), from Jeju Island, Korea

Taekjun Lee[1,2], Sook Shin[2,3,*]

[1]Division of Life Sciences, College of Life Sciences and Biotechnology, Korea University, Seoul 02841, Korea
[2]Marine Biological Resource Institute, Sahmyook University, Seoul 01795, Korea
[3]Department of Chemistry Life Science, Sahmyook University, Seoul 01795, Korea

ABSTRACT

The brittle star, *Amphistigma minuta* H.L. Clark, 1938, was collected from Munseom Island, Jeju-do, Korea, by SCUBA diving. This species has distinct morphological features, as follows: a small disk with large papillae on the disk margin; slender arms and four arm spines at proximal; and oral parts the same as those of the genus *Amphipholis*. Additionally, we obtained partial sequences of the cytochrome *c* oxidase subunit I gene (COI) (513 bp) and compared them with sequences from Australian *A. minuta* and 13 other species of Amphiuridae. As a result, intraspecific pairwise distance was 0.4% between two Korean individuals and intraspecific distance between the Australian and the Korean populations was 2.6–2.8%, which indicates they should be considered as the same species. Ultimately, 15 species of the family Amphiuridae have currently been recorded in Korea, including *A. minuta*.

Keywords: Echinodermata, morphology, ophiuroids, taxonomy, COI

INTRODUCTION

The family Amphiuridae Ljungman, 1867 is the most diverse taxon in the suborder Gnathophiurina Matsumoto, 1915 and includes 26 existing genera (Stöhr et al., 2019). According to Shin (2012), 14 species belonging to five genera among the Amphiuridae have been recorded in Korea. The genus *Amphistigma* H.L. Clark, 1938 comprises two species: *A. minuta* H.L. Clark, 1938 and *A. watsonae* Baker, 1979. These two ophiuroids are rarely collected and have only been reported to occur in the Tasman Sea and the waters surrounding Australia (Baker, 1979).

DNA barcoding has proven to be an effective tool for species identification in ophiuroids and other echinoderms (Ward et al., 2008; Hoareau and Boissin, 2010; Uthicke et al., 2010; Layton et al., 2016; Boissin et al., 2017). DNA barcoding employs sequence variation in a gene or genes, such as the 658 bp region of the mitochondrial cytochrome *c* oxidase subunit 1 (COI) gene, as a tool for specimen identification and species discovery (Hebert et al., 2003). Furthermore, field surveys coupled with DNA barcoding have proved useful for rapidly assessing biodiversity (Hebert et al., 2003; Miller et al., 2016), and an integrative approach to taxonomy has emerged as a powerful and necessary means for assessing species boundary and species diversity (Puillandre et al., 2012). According to a previous ophiuroid DNA barcoding study (Boissin et al., 2017), the Kimura 2-parameter (K2P) interspecific distance within the class Ophiuroidea has been reported to range from 5.6% to 31.6%, with a mean of 18.9%, and intraspecific distance ranged from 0.5% to 6.4% with a mean of 2.2%.

In this study, we redescribed the brittle star, *A. minuta*, with detailed figures produced by scanning electron microscopy (SEM). In addition, we obtained partial COI sequences and uploaded them to the GenBank database.

The brittle stars were collected from Munseom Island, which is located on the southern coast of Jeju-do, Korea. Samples were collected by SCUBA diving, separated from bryozoans, then immediately preserved in ethyl alcohol solution (>95%). The samples were deposited at the Marine

*To whom correspondence should be addressed
Tel: 82-2-3399-1723, Fax: 82-2-3399-1729
E-mail: shins@syu.ac.kr

eISSN 2234-8190

Table 1. Pairwise distance values (%) based on 513 bp mitochondrial COI sequences, calculated using the Kimura 2-parameter method with 1,000 bootstrap replicates

	Species	GenBank accession No.	Pairwise distance (%)																	
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	*Amphistigma minuta 1* (Korea)	MK612076																		
2	*Amphistigma minuta 2* (Korea)	MK612077	0.4																	
3	*Amphistigma minuta* (Australia)	KU895015	2.6	2.8																
4	*Amphipholis squamata*	NC_013876	16.8	16.8	16.0															
5	*Amphiura latisquama*	KU895020	26.2	26.8	26.8	25.3														
6	*Amphiura joubini*	KU895004	26.4	27.0	27.9	25.6	19.6													
7	*Amphiura (A.) poecila*	KU895035	24.8	25.4	25.1	27.6	23.7	21.0												
8	*Amphiura (A.) stictacantha*	KU895036	30.4	31.0	30.2	28.4	24.2	28.0	17.7											
9	*Amphiura (F.) octacantha*	KU895025	25.4	25.7	25.7	26.5	25.3	24.8	25.3	29.6										
10	*Amphiura (O.) dikellacantha*	KU895018	28.2	28.2	27.1	27.0	20.0	23.2	23.9	26.0	31.2									
11	*Ophiocentrus inaequalis*	KU895053	26.3	26.9	26.6	25.0	23.3	24.0	26.4	25.9	26.2	24.6								
12	*Ophiocentrus pilosus*	KU895049	22.7	23.3	23.8	22.5	21.8	23.3	23.7	24.3	23.7	25.4	17.6							
13	*Ophiodaphne formata*	KU895055	22.3	22.8	23.4	21.5	25.8	29.0	26.9	26.8	25.6	29.1	24.3	26.0						
14	*Ophiodaphne scripta*	KU895056	24.6	24.7	23.3	23.2	26.7	29.5	27.4	25.9	27.0	24.6	26.4	26.4	20.5					
15	*Ophiophragmus filograneus*	KU895058	24.4	24.9	22.7	24.8	24.8	25.4	27.5	27.6	26.3	24.0	24.3	21.9	24.6	24.1				
16	*Ophiophragmus wurdemanii*	KU895059	23.8	24.3	24.0	24.0	25.7	24.4	24.5	29.3	23.2	23.5	23.7	21.6	21.1	23.8	19.9			
17	*Astroceras annulatum*	AB758778	27.3	27.9	27.0	28.1	27.8	27.6	25.9	29.6	25.8	30.4	23.7	23.7	28.3	31.1	27.2	28.7		
18	*Asteromorpha capensis*	AB758754	27.0	27.6	25.6	27.6	26.8	26.9	28.2	30.3	29.3	30.8	25.1	19.6	29.2	31.1	25.4	24.6	20.1	

Echinoderm Resources Bank of Korea (MERBK). The specimens were lightly bleached following the methods of Stöhr et al. (2012b), before being examined by SEM. Bleached specimens were dried in an oven for 6 h at 55°C, then photographs were taken using the JSMmicroscope 6510 SEM (JEOL, Tokyo, Japan).

The total genomic DNA was extracted from the podia tissue of Korean *A. minuta* following the DNeasy kit protocol (Qiagen, Hilden, Germany). A COI fragment with a length of approximately 540 base pairs (bp) was amplified using the primer pair OphiF1 (Driskell, unpublished) and COIceR (Hoareau and Boissin, 2010). The PCR cycle initiated denaturation at 95°C (8 min), which was followed by a sequence of 95°C (30 s), 50°C (45 s), and 72°C (60 s) for 40 cycles, finishing with a final elongation at 72°C (12 min). PCR product quality was assessed by electrophoresis using a 1.0% agarose gel and the NanoDrop 1000 (Thermo Scientific, Waltham, MA, USA). PCR products were sequenced using ABI BigDye Terminator kits (Applied Biosystems, Foster City, CA, USA) on the ABI 3730XL DNA Analyzer. To construct a neighbor joining tree, we used two sequences from Korean *A. minuta* and 16 ophiuroid sequences from GenBank, including 14 species of Amphiuridae and two euryalids as outgroups (Perseke et al., 2010; Okanishi and Fujita, 2013; Hugall et al., 2016): *Astroceras annulatum* Mortensen, 1933 (GenBank accession No. AB758778) and *Asteromorpha capensis* Mortensen, 1925 (GenBank accession No. AB758754) (Table 1). Pairwise genetic distances based on p-distances were calculated using MEGA7 (Kumar et al., 2016) and gaps and/or missing data were treated as pairwise deletions. In order to compare our results to data from the literature, K2P distances were also calculated. Neighbor-joining (NJ) analysis (Saitou and Nei, 1987) based on p-distances with 1,000 non-parametric bootstrap replicates (Felsenstein, 1985) was performed using MEGA7 (Kumar et al., 2016).

SYSTEMATIC ACCOUNTS

Class Ophiuroidea Gray, 1840

[1]*Superorder Ophintegrida O'Hara, Hugall, Thuy, Stöhr and Martynov, 2017

[2]*Order Amphilepidida O'Hara, Hugall, Thuy, Stöhr and Martynov, 2017

Suborder Gnathophiurina Matsumoto, 1915

Superfamily Amphiuroidea Ljungman, 1867

Family Amphiuridae Ljungman, 1867

Korean name: [1]*전사미상목(신칭), [2]*양린사미목(신칭)

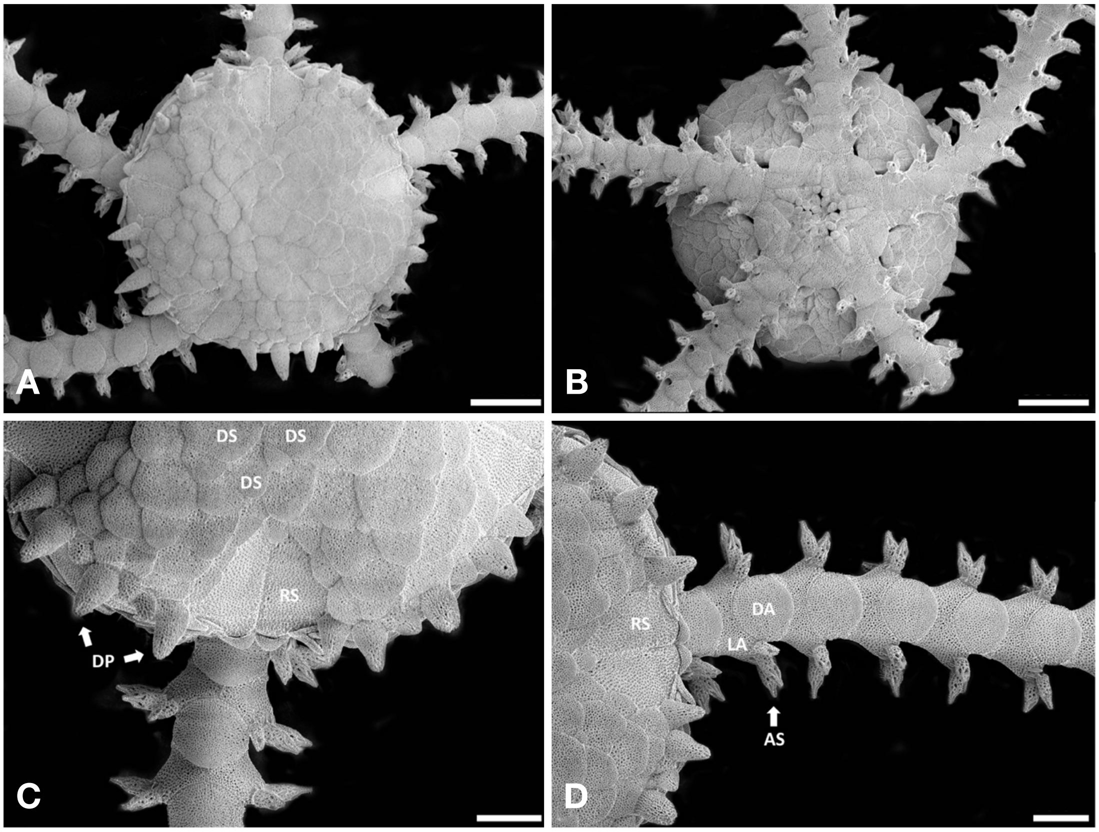

Fig. 1. Scanning electron microscopy images of *Amphistigma minuta* H.L. Clark, 1938. A, Dorsal side; B, Ventral side; C, Radial shields, disk scales, and disk papillae; D, Disk margin and dorsal side of arm. AS, arm spine; DA, dorsal arm plate; DP, disk papilla; DS, disk scale; LA, lateral arm plate; RS, radial shield. Scale bars: A, B = 500 μm, C, D = 200 μm.

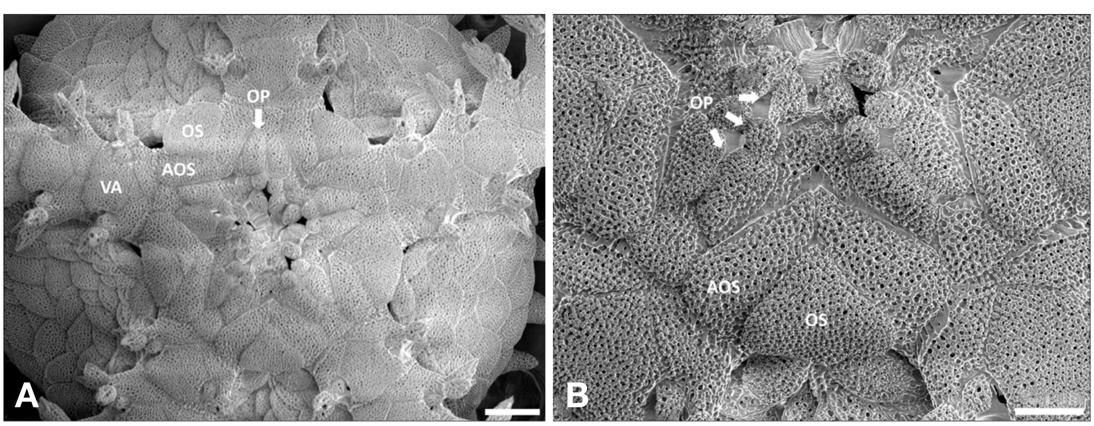

Fig. 2. Scanning electron microscopy images of *Amphistigma minuta* H.L. Clark, 1938. A, Oral frame and ventral side of proximal arm; B, Oral frame. AOS, adoral shield; OP, oral papilla; OS, oral shield; VA, ventral arm plate. Scale bars: A = 200 μm, B = 100 μm.

[1]*Genus *Amphistigma* H.L. Clark, 1938

[2]****Amphistigma minuta* H.L. Clark, 1938**

Amphistigma minuta HL Clark, 1938: 245; AM Clark, 1970: 33; Baker, 1978: 47; Stöhr et al., 2019: 242886.

Korean name: [1]*뿔양편거미불가사리속(신칭), [2]*작은뿔양편거미불가사리(신칭)

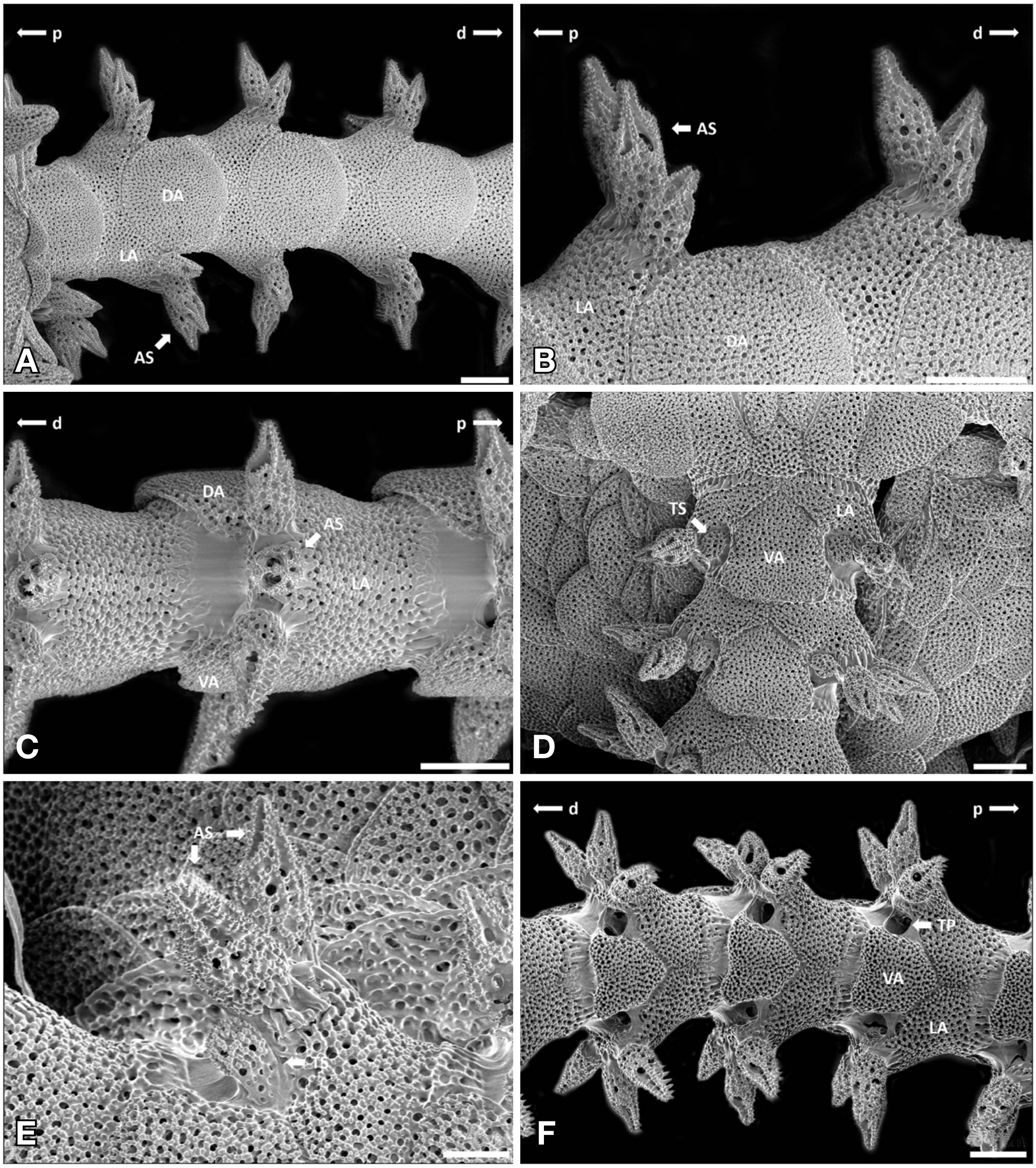

Fig. 3. Scanning electron microscopy images of *Amphistigma minuta* H.L. Clark, 1938. A, Dorsal side of arm; B, Arm spines and part of dorsal side of arm; C, Lateral side of arm; D, Ventral side of arm and disk; E, Tentacle scale and arm spines; F, Ventral side of distal arm. AS, arm spine; d, distal; DA, dorsal arm plate; LA, lateral arm plate; p, proximal; TP, tentacle pore; TS, tentacle scale; VA, ventral arm plate. Scale bars: A–D, F=100 μm, E=50 μm.

Material examined. 10 specimens, Korea: Jeju-do: Munseom Island (33°13′38.2″N, 126°33′47.4″E), 27 Nov 2018, depth of 35.7 m, SCUBA diving, Lee T; eight specimens, Munseom Island (33°13′38.7″N, 126°33′47.3″E), 28 Nov 2018, depth of 28.8 m, SCUBA diving, Lee T.

Description. Disk small, round (diameter: 1.5–2.4 mm) (Fig. 1A, B), covered with well-defined scales (Fig. 1A, C). Large disk papillae, tapered to the tip with dulled single point situated at disk margin (Fig. 1C). Five arms, slender, four to five times longer than disk diameter. Radial shields relatively large, with length approximately a quarter of disk diameter and rounded triangular form, closely appressed to the radial line. Oral shield small, triangular form, wider than long with rather sharp proximal angle (Fig. 2A, B). Adoral shield large, slightly over two times wider than long, proximally and distally separated by oral shield (Fig. 2B). Three oral

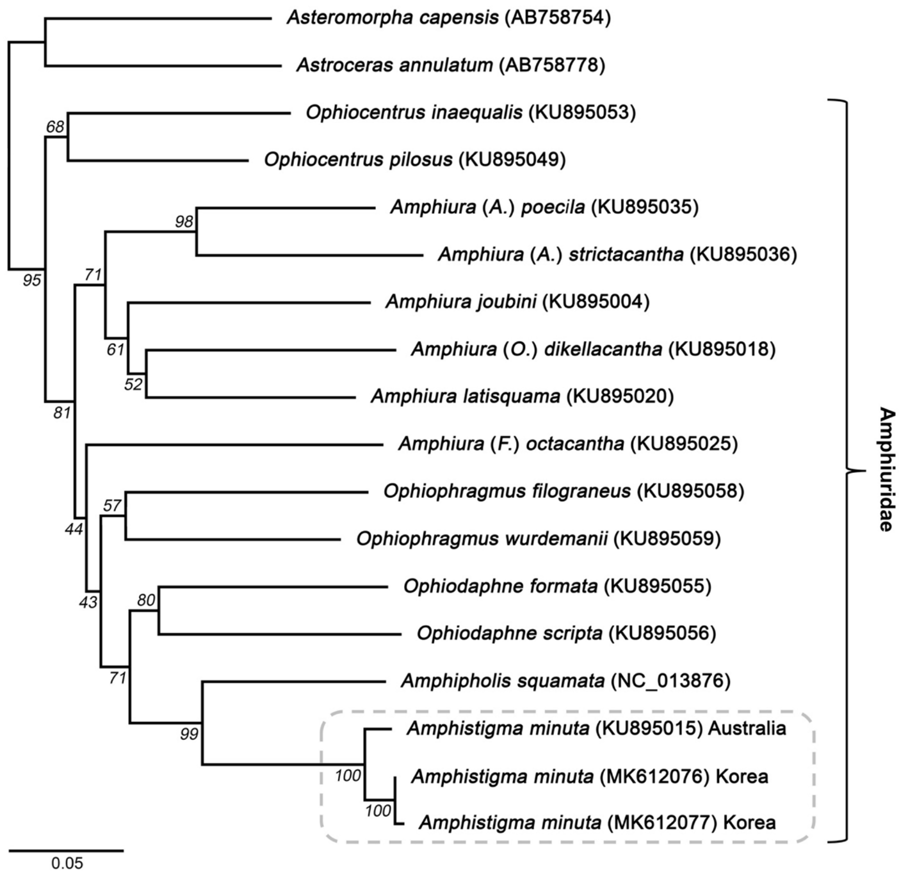

Fig. 4. Neighbor joining analysis of 513 bp COI sequences based on p-distance with 1,000 non-parametric bootstrap replicates. The values of bootstrap support are indicated on each node as ≤100.

papillae on each side of the jaw, with distal the largest, and twice as long as others (Fig. 2B). No oral tentacle scale (Fig. 2B). Dorsal arm plate ovate, slightly wider than long (Fig. 3A). Lateral arm plate wider than long, similar in length to dorsal arm plate (Fig. 3A–C), and spine articulation same as that of amphiurid form. Ventral arm plate longer than wide and concaved in the middle of lateral margin by tentacle pore (Fig. 3D, F). Arm spines four to three from proximal to distal, short, stout, pointed (Fig. 3A–C), and ventral-most arm spine thorny and slightly larger than others (Fig. 3C). Tentacle scale single, ovate, relatively large (Fig. 3D, E), and tentacle pore naked from fourth or fifth ventral plate (Fig. 3F).

Habitat. The species has been reported to occur on algae in the shallow waters of the Tasman Sea and the waters surrounding Australia, at a depth range of 2–15 m (Baker, 1979). We collected *A. minuta* at a depth of 28–33 m from Munseom Island, Jeju-do, Korea, which had a water temperature of 20°C here, and the brittle stars occurred on the bryozoan *Bantariella bocki* (Silén, 1942), which covered several black corals at this site.

Distribution. Korea (Jeju Island), Tasman Sea, and Southern/Western Australia.

Remarks. This species has been recorded in the restricted regions as the Tasman Sea and the adjacent waters of Australia (Clark, 1938; Clark, 1970; Baker, 1979). To the best of our knowledge, this study is the first to report *A. minuta* in the northern Pacific region and is the deepest collection record for this species.

Our *A. minuta* specimens displayed some morphological and molecular differences to Australian *A. minuta*. Previous studies have recorded that *A. minuta* had three arm spines proximally (Clark, 1938), but our specimens have four, which was the only morphological difference observed between Australian and Korean *A. minuta*. Moreover, we compared mitochondrial COI sequences (513 bp) from Korean and Australian *A. minuta*. The pairwise distance values highlighted a 2.6–2.8% difference between the Korean and Australian *A. minuta* (Table 1). These values were lower than average compared to interspecific distances in previous

ophiuroid DNA barcoding studies (18.9%), and these values were similar to the average intraspecific distance within ophiuroids (2.2%) (Boissin et al., 2017). Furthermore, the results of the NJ analysis confirmed that Korean and Australian *A. minuta* form a distinct monophyletic clade, which presented a close relationship with *Amphipholis squamata* Ljungman, 1867 (Fig. 4). The class Ophiuroidea is a diverse and abundant taxon within the echinoderms, occurring from the equator to the poles and from coastal shores to hadal trenches (Stöhr et al., 2012a). The Indo-Pacific region has the highest ophiuroid species richness overall (Stöhr et al., 2012a), and within this region, some ophiuroid species inhabited Korea and Southern Japan (Rowe and Gates, 1995; Shin, 2012). Accordingly, even though our specimens were found far from their region of origin (Australia), we consider that Korean *A. minuta* are the same species as Australian *A. minuta*.

ACKNOWLEDGMENTS

This study was supported by the Marine Biotechnology Program of the Korea Institute of Marine Science and Technology Promotion (KIMST), which was funded by the Ministry of Oceans and Fisheries (MOF) (No. 20170431). It was also supported by a grant from the National Institute of Biological Resources (NIBR), which was funded by the Ministry of Environment of the Republic of Korea (NIBR201902107).

REFERENCES

Baker AN, 1979. Some Ophiuroidea from the Tasman Sea and adjacent waters. New Zealand Journal of Zoology, 6:21-51. https://doi.org/10.1080/03014223.1979.10428345

Boissin E, Hoareau TB, Paulay G, Bruggemann JH, 2017. DNA barcoding of reef brittle stars (Ophiuroidea, Echinodermata) from the southwestern Indian Ocean evolutionary hot spot of biodiversity. Ecology and Evolution, 7:11197-11203. https://doi.org/10.1002/ece3.3554

Clark AM, 1970. Notes on the family Amphiuridae (Ophiuroidea). Bulletin of the British Museum of Natural History (Zoology), 19:1-81. https://doi.org/10.5962/bhl.part.24085

Clark HL, 1938. Echinoderms from Australia, an account of collections made in 1929 and 1932. Memoirs of the Museum of Comparative Zoology at Harvard College, 55:1-597.

Felsenstein J, 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution, 39:783-791. https://doi.org/10.1111/j.1558-5646.1985.tb00420.x

Hebert PDN, Cywinska A, Ball SL, deWaard JR, 2003. Biological identifications through DNA barcodes. Proceedings of the Royal Society of B: Biological Sciences, 270:313-321. https:// doi.org/10.1098/rspb.2002.2218

Hoareau TB, Boissin E, 2010. Design of phylum-specific hybrid primers for DNA barcoding: addressing the need for efficient COI amplification in the Echinodermata. Molecular Ecology Resources, 10:960-967. https://doi.org/10.1111/j.1755-0998.2010.02848.x

Hugall AF, O'Hara TD, Hunjan S, Nilsen R, Moussalli A, 2016. An exon-capture system for the entire class Ophiuroidea. Molecular Biology and Evolution, 33:281-294. https://doi.org/10.1093/molbev/msv216

Kumar S, Stecher G, Tamura K, 2016. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution, 33:1870-1874. https://doi.org/10.1093/molbev/msw054

Layton KKS, Corstorphine EA, Hebert PDN, 2016. Exploring Canadian echinoderm diversity through DNA barcodes. PLoS ONE, 11:e0166118. https://doi.org/10.1371/journal.pone.0166118

Miller SE, Hausmann A, Hallwachs W, Janzen DH, 2016. Advancing taxonomy and bioinventories with DNA barcodes. Philosophical Transactions of the Royal Society B: Biological Sciences, 371:20150339. https://doi.org/10.1098/rstb.2015.0339

Okanishi M, Fujita T, 2013. Molecular phylogeny based on increased number of species and genes revealed more robust family-level systematics of the order Euryalida (Echinodermata: Ophiuroidea). Molecular Phylogenetics and Evolution, 69:566-580. https://doi.org/10.1016/j.ympev.2013.07.021

Perseke M, Bernhard D, Fritzsch G, Brümmer F, Stadler PF, Schlegel M, 2010. Mitochondrial genome evolution in Ophiuroidea, Echinoidea, and Holothuroidea: insights in phylogenetic relationships of Echinodermata. Molecular Phylogenetics and Evolution, 56:201-211. http://dx.doi.org/10.1016/j.ympev.2010.01.035

Puillandre N, Lambert A, Brouillet S, Achaz G, 2012. ABGD, Automatic Barcode Gap Discovery for primary species delimitation. Molecular Ecology, 21:1864-1877. https://doi.org/10.1111/j.1365-294X.2011.05239.x

Rowe FWE, Gates J, 1995. Echinodermata. In: Zoological Catalogue of Australia, Vol. 33 (Ed., Wells A). CSIRO, Melbourne, pp. 1-510.

Saitou N, Nei M, 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular Biology and Evolution, 4:406-425. https://doi.org/10.1093/oxfordjournals.molbev.a040454

Shin S, 2012. Brittle star I: Echinodermata: Asterozoa: Ophiuroidea. Invertebrate fauna of Korea, Vol. 32, No. 3. National Institute of Biological Resources, Incheon, pp. 1-143.

Stöhr S, O'Hara TD, Thuy B, 2012a. Global diversity of brittle stars (Echinodermata: Ophiuroidea). PLoS ONE, 7:e31940. https://doi.org/10.1371/journal.pone.0031940

Stöhr S, Sautya S, Ingole B, 2012b. Brittle stars (Echinodermata: Ophiuroidea) from seamounts in the Andaman Sea (Indian Ocean): first account, with descriptions of new spe-

cies. Journal of the Marine Biological Association of the United Kingdom, 92:1195-1208. https://doi.org/10.1017/S0025315412000240

Stöhr S, O'Hara T, Thuy B, 2019. World Ophiuroidea database [Internet]. World Register of Marine Species, Accessed 3 Mar 2019, <http://www.marinespecies.org/aphia.php?p=taxdetails&id=123084>.

Uthicke S, Byrne M, Conand C, 2010. Genetic barcoding of commercial Beche-de-mer species (Echinodermata: Holothuroidea). Molecular Ecology Resources, 10:634-646. https://doi.org/10.1111/j.1755-0998.2009.02826.x

Ward RD, Holmes BH, O'Hara TD, 2008. DNA barcoding discriminates echinoderm species. Molecular Ecology Resources, 8:1202-1211. https://doi.org/10.1111/j.1755-0998.2008.02332.x

Received March 15, 2019
Revised April 15, 2019
Accepted April 16, 2019